Full Length Research Paper

Emergence of antimicrobial resistance in vibrio cholerae of North India

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Infectious diarrheal diseases are responsible for considerable morbidity and mortality, especially in developing countries. The emerging resistance to many antibiotics is a matter of great concern since in the recent past antibacterial resistance in *V. cholerae* was seldom documented. The emergence of considerable resistance in a short span of time is needed to be addressed, detected, documented and controlled. The present study was done to evaluate the changing pattern of antimicrobial sensitivity pattern in *V.cholerae* isolated from the stool specimens from diarrhea patients. A total of 386 Stool specimens were processed between 2006 and August 2011 from diarrhea patients admitted to the Jawaharlal Nehru Medical College and Hospital (JNMCH), Aligarh. The specimens were cultured on to MacConkey agar, Thiosulphate-citrate bile salt sucrose (TCBS), Blood agar and inoculated in alkaline peptone water. Isolates were identified as *V. cholerae* according to the recommended standard laboratory methods and confirmed by serology using antisera. Antimicrobial susceptibility was carried out by Kirby-bauer disc diffusion method. Out of 386 faecal specimens examined from the patients of diarrhea, 66 were positive for *V. cholerae*. All the isolates during the years 2006 to 2011 depicted a constant increase in resistance to nalidixic acid, ciprofloxacin, tetracyline, doxycycline and cotrimoxazole with 100% resistance to nalidixic acid and cotrimoxazole in some isolates. The isolates remained consistently sensitive to gentamycin, cefotaxime and piperacillin.

Keywords: Diseases, alkaline, antibiotics, *V.cholerae*, antimicrobial, nalidixic acid.

INTRODUCTION

Vibrio cholerae (V. cholerae) 01 the causative organism of cholera was first described by Robert Koch in1883 (Sakazaki et al., 1992). Of recent, *V. cholerae* 0139 has also been associated with severe forms of cholera (Albert et al., 1994). Notification and surveillance systems of infectious diseases including that caused by *V. cholerae* 01 in most developing countries is lacking, but available epidemiological and clinical evidence show that cholera is a public health problem. Throughout history, *V. cholerae* 0l has caused repeated epidemics in various areas of the world, especially in Asia, the Middle East and Africa

(WHO,1997).Cholera, caused by toxigenic strains of *V. cholerae* O1 or O139, continues to be a major cause of illness and death, particularly in developing countries. Treatment consists of early administration of rehydration therapy with appropriate oral or intravenous fluids. The World Health Organization recommends antimicrobial drug treatment for severely dehydrated patients with suspected cholera because it substantially shortens the duration of diarrhea by reducing the volume of watery stools, decreases fluid requirements, and limits transmission by decreasing fecal excretion of *V.cholerae* (WHO, 2005). Like most bacteria of clinical and public health significance, *V.cholerae* is continuously becoming more resistant to a variety of antimicrobial agents, necessitating the use of newer drugs which are expensive and have more adverse effects to patients. The emerging and progressive increase in resistance to

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Table 1. Distribution of bacterial isolates in study group

S.No.	Bacterial isolates	No. of cases (%)
1	Escherichia coli	233 (60)
2	Klebsiella spp.	70 (18)
3	V. cholera	66 (17)
4	Shigella spp.	08 (.02)
5	Salmonella spp.	05 (.01)
6	Citrobacter spp.	04 (.01)

Table 2. Distribution of vibrio cholera according to serotyping

Year	Ogawa	Inaba	Hikojima
2011	18	05	-
2010	09	01	-
2009	12	01	-
2008	08	-	-
2007	07	-	-
2006	05	-	-

multiple drugs among strains causing clinical cases of cholera in developing countries is becoming a serious concern as in the recent past antibacterial resistance in *V. cholerae* was seldom documented. The emergence of considerable resistance in a short span of time is needed to be addressed, detected, documented and controlled. So, this study was conducted with following aims and objectives:

1. To estimate the prevalence of *V. cholerae* among patients presenting with diarrhea to JN Medical College, Aligarh.

2. To identify and serotype *V. cholera* by using standard biochemical tests and specific antisera.

3. To study the changing patterns of antimicrobial susceptibility in *V. cholerae.*

MATERIAL AND METHODS

Study group comprised of 386 patients who presented with symptoms of cholera like diarrhea to JN Medical College and Hospital (JNMCH), Aligarh between January 2006 and August 2011 .Stool specimens were transported immediately in Cary Blair medium to Enteric Laboratory of Department of Microbiology, JNMCH. Specimens were microscopically examined by Gram staining and motility for characteristic shape and darting motility. Specimens were cultured on MacConkey Agar, Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS) and Blood Agar (BA). They were also inoculated in Alkaline Peptone Water (APW) and Selenite F broth. APW was examined after 6 hours of incubation for characteristic motility of *V. Cholera* and blind subculture was done in another APW. Subculture was done from APW on TCBS and after overnight incubation plates were incubated at 37ºC for 24 hours. The isolates obtained were tested biochemically and confirmed by slide agglutination with polyvalent O1 and monovalent Ogawa and Inaba antiserum.

Antimicrobial drug susceptibility testing was performed by using the Kirby-bauer disk diffusion technique on Mueller-Hinton agar with commercial disks (HI-MEDIA) using following antimicrobial agents: Quinolones- ciprofloxacin (30µg), nalidixic acid (30µg), Aminoglycosides-gentamicin (10µg), tobramycin (30µg), amikacin (30µg), Cephalosporins-ceftriaxone (30µg), cefotaxime (30µg), cefoperazone+salbactam (75µg),tetracycline (10µg), doxycycline (30µg), cotrimaxazole (1.25/23.75µg), piperacllin (100µg).

RESULTS

During the January 2006 – August 2011 study period, we isolated 66 (17%) *V.cholerae* O1 organisms (Table 1), among which 59 (89.4%) were Ogawa and 7 (10.6%) were Inaba serotypes (Table 2). Comparison of the antimicrobial susceptibility patterns among *V. cholerae* 01

Table 3. Antimicrobial senstivity pattren of vibrio cholera isolates

ANTIMICROBIAL AGENT	2006 n=13(%)	2007 n=15(%)	2008 n=06(%)	2009 n=15(%)	2010 n=12(%)	2011 n=05(%)	TOTAL RESISTANT 2006-2011 (n=66)
Nalidixic acid	11 (84.6)	12 (80)	5 (83.3)	13 (86.6)	12 (100)	05 (100)	58 (87.8)
Ciprofloxacin	01 (7.6)	01 (6.6)	01 (16.6)	02 (13.3)	01 (8.3)	01 (20)	07 (10.6)
Tetracycline	01 (7.6)	02 (13.3)	01 (16.6)	03 (30.3)	01 (8.3)	01 (20)	09 (13.6)
Doxycycline	01 (7.6)	02 (13.3)	01 (16.6)	02 (13.3)	01 (8.3)	01 (20)	08 (12.1)
Cotrimoxazole	09 (69.2)	13 (86.6)	05 (83.3)	15 (100)	12 (100)	05 (100)	59 (89.3)
Gentamycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Piperacillin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cefotaxime	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

isolates is discussed in Table 3. In the study period, a sudden upsurge in resistance to cotrimoxazole has eventually led to its 100 percent resistance in 2011 among *V. cholerae* isolates, from 69.2% in 2006 to 86.6% in 2007 before decreasing to 83.3% in 2009. An increase in resistance to nalidixic acid was also observed during the same period from 84.6% in 2006 to 86.6% in 2009 and eventually to 100 percent resistance in 2011 before decreasing to 80% in 2007 and 83.3% in 2008. Resistance to tetracycline was 7.6% in 2006, increasing to 30.3% in 2009 and eventually to 20% in 2011. Ciprofloxacin and doxycycline also showed resistance in 7.6% cases in 2006 increasing to 16.6% in 2008 and 20% in 2011. Although the V.cholera isolates remains constantly sensitive to gentamycin, cefotaxime and piperacillin.

DISCUSSION

Resistance to commonly used antimicrobial drugs represents a critical public health problem because it complicates treatment and may result in longer hospital stays for patients. In addition, most of the population in developing countries cannot afford the newer and more expensive drugs(Bhattacharya et al., 2011).The problem of antimicrobial resistance in organisms causing diarrheal diseases will continue to be ongoing in both developed and developing countries (Murray et al,1994). Both in India and worldwide, there is great variation in the distribution of antibiotic resistant *V. cholerae* 01strains (Hofer et al, 1999). In the present study, 100% resistance for co-trimoxazole and nalidixic acid has been observed among *V. cholerae* isolates. Resistance to co-trimoxazole was similarly reported in a study by Inacio et al, 2007 and Folgaso et al., 2001. The high incidence of resistance is alarming because co-trimoxazole is widely used to prevent opportunistic infections in HIV patients (Chintu et al., 2004) and sulfadoxine/pyrimethamine, a related antimicrobial that has been demonstrated to produce cross-resistance, is commonly used for malaria treatment. Similar significant increase in resistant strains was observed against nalidixic acid by Urassa et al, 2000. Tetracycline is the drug of choice for treating cholera (WHO, 2005); however, during the 6-year period, we observed the emergence of tetracycline resistance among *V. cholerae* O1 isolates and a sudden upsurge in such resistance in 2009 when 30% of the isolates were resistant. However tetracycline resistance reported by Mhalu et al., (*3*), from an epidemic of cholera in Tanzania, and Bhattacharya et al., 2011 was on the higher side (76%). Notably, tetracycline-resistant *V.cholerae* O1 strains have also been responsible for major epidemics in Latin America, Tanzania, Bangladesh, and Zaire (Garg et al., 2000). Alternative drugs, such as the newer fluoroquinolones, possess excellent activity against *V. cholera* O1 and O139 serogroups. However, increased resistance to newer fluoroquinolones, such as ciprofloxacin and norfloxacin, among *V. cholerae* strains belonging to O1 serogroup has also been reported (Garg et al, 2001). Vibrio strains resistant to fluoroquinolones have been reported previously, especially in Asia, and a continuous rise in the incidence of strains resistant to quinolones among diarrhoea-causing pathogens during the last few years has been observed in Asia (Sarkar et al., 2003).

CONCLUSION

Our observations emphasize the need for continuous surveillance of antimicrobial drug susceptibility patterns of *V. cholerae*. Providing early information on antimicrobial drug susceptibility to practitioners in affected areas will lessen the burden of illness and death from this devastating disease.

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